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Special Article

SHATTUCK LECTURE — NEURODEGENERATIVE DISEASES AND PRIONS

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TWENTY-FIVE years ago, little was known about the causes of neurodegenerative diseases. Now, however, it is clear that they result from abnormalities in the processing of proteins. In each of these diseases, defective processing causes the accumulation of one or more specific neuronal proteins.

Of all the laboratory research on neurodegenerative diseases, the studies that led to the discovery of prions have yielded the most unexpected findings. The idea that a protein can act as an infectious pathogen and cause degeneration of the central nervous system was accepted only after a long and arduous battle.[1] The concept of prions not only has provided an explanation of how a disease can be both infectious and genetic, but has also revealed hitherto unknown kinds of neurologic diseases. This review presents a unifying concept of degenerative brain diseases, based on what we have learned about prions.[2]

Alzheimer's disease is the most common neurodegenerative disorder (Table 1). In the United States, approximately 4 million people have Alzheimer's disease, and approximately 1 million have Parkinson's disease.[3-5] Much less common are amyotrophic lateral sclerosis, frontotemporal dementia, prion diseases, Huntington's disease, and spinocerebellar ataxias.

With the increase in life expectancy, there has been concern about the incidence of Alzheimer's and Parkinson's diseases. Among persons who are 60 years old, the prevalence of Alzheimer's disease is approximately 1 in 10,000, but among those who are 85 years old, it is greater than 1 in 3.[6] These data suggest that by 2025, there will be more than 10 million cases of Alzheimer's disease in the United States, and by 2050, the number will approach 20 million.[4] The annual cost associated with Alzheimer's disease in the United States is estimated at $200 billion. Age is also the most important risk factor for Parkinson's disease. Nearly 50 percent of persons who are 85 years old also have at least one symptom or sign of parkinsonism.[7]

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TABLE 1. PREVALENCE OF NEURODEGENERATIVE DISEASES IN THE UNITED STATES IN 2000.

DISEASE	NO. OF CASES	NO. PER 100,000 POPULATION*
Prion disease	400	<1
Alzheimer's disease	4,000,000	1450
Parkinson's disease	1,000,000	360
Frontotemporal dementia	40,000	14
Pick's disease	5,000	2
Progressive supranuclear palsy	15,000	5
Amyotrophic lateral sclerosis	20,000	7
Huntington's disease	30,000	11
Spinocerebellar ataxias	12,000	4

*Data are based on a population of approximately 275 million in 2000.

Virtually all neurodegenerative disorders involve abnormal processing of neuronal proteins. The aberrant mechanism can entail a misfolding of proteins, altered post-translational modification of newly synthesized proteins, abnormal proteolytic cleavage, anomalous gene splicing, improper expression, or diminished clearance of degraded protein. Misprocessed proteins often accumulate because the cellular mechanisms for removing them are ineffective. The particular protein that is improperly processed determines the malfunction of distinct sets of neurons and thus the clinical manifestations of the disease.

PRIONS

Prions are infectious proteins. In mammals, prions reproduce by recruiting normal cellular prion protein (PrP^C) and stimulating its conversion to the disease-causing (scrapie) isoform (PrP^{Sc}). A major feature that distinguishes prions from viruses is that PrP^{Sc} is encoded by a chromosomal gene.[8] Limited proteolysis of PrP^{Sc} produces a smaller, protease-resistant molecule of approximately 142 amino acids, designated PrP 27–30, which polymerizes into amyloid.[9]

The polypeptide chains of PrP^C and PrP^{Sc} are identical in composition but differ in their three-dimensional, folded structures (conformations). PrP^C is rich in α-helixes (spiral-like formations of amino acids) and has little β-sheet (flattened strands of amino acids), whereas PrP^{Sc} is less rich in α-helixes and has much more β-sheet.[10] There is evidence that PrP^{C} has three α-helixes and two short β-strands; in contrast, a plausible model suggests that PrP^{Sc} may have only two α-helixes and more β-strands (Fig. 1).[11,12] This structural transition from α-helixes to β-sheet in PrP is the fundamental event underlying prion diseases.

Four new concepts have emerged from studies of prions. First, prions are the only known example of infectious pathogens that are devoid of nucleic acid. All other infectious agents possess genomes composed of either RNA or DNA that direct the synthesis of their progeny. Second, prion diseases may be manifested as infectious, genetic, or sporadic disorders. No other group of illnesses with a single cause has such a wide spectrum of clinical manifestations. Third, prion diseases result from the accumulation of PrP^{Sc}, which has a substantially different conformation from that of its precursor, PrP^{C}. Fourth, PrP^{Sc} can have a variety of conformations, each of which seems to be associated with a specific disease. How a particular conformation of PrP^{Sc} is imparted to PrP^{C} during replication in order to produce a nascent PrP^{Sc} with the same conformation is unknown. The factors that determine the site in the central nervous system where a particular PrP^{Sc} is deposited are also not known.

PRION DISEASES

Prion diseases have a broad spectrum of clinical manifestations, including dementia, ataxia, insomnia, paraplegia, paresthesias, and deviant behavior.[13] Neuropathological findings range from an absence of atrophy to widespread atrophy, from minimal to widespread neuronal loss, from sparse to widespread vacuolation or spongiform changes, from mild to severe reactive astrocytic gliosis, and from an absence of PrP amyloid plaques to an abundance of plaques.[14] None of these findings except the presence of PrP amyloid plaques is unequivocally diagnostic of a prion disease.

The sporadic form of Creutzfeldt–Jakob disease, which is typically manifested as dementia and myoclonus, accounts for approximately 85 percent of all cases of prion disease in humans, whereas infectious and inherited prion diseases account for the rest. Familial Creutzfeldt–Jakob disease, Gerstmann–Sträussler–Scheinker disease, and fatal familial insomnia are all dominantly inherited prion diseases caused by mutations in the prion protein gene (*PRNP*) (Table 2).[15-19] Experiments that showed transmission of these diseases by filtrates of brain from familial cases[20,21] were wrongly attributed to a virus. There is no Creutzfeldt–Jakob disease virus, and familial prion diseases are caused by mutations in *PRNP*.[22]

Epidemiologic Features

Prions cause Creutzfeldt–Jakob disease in humans throughout the world. The incidence of sporadic

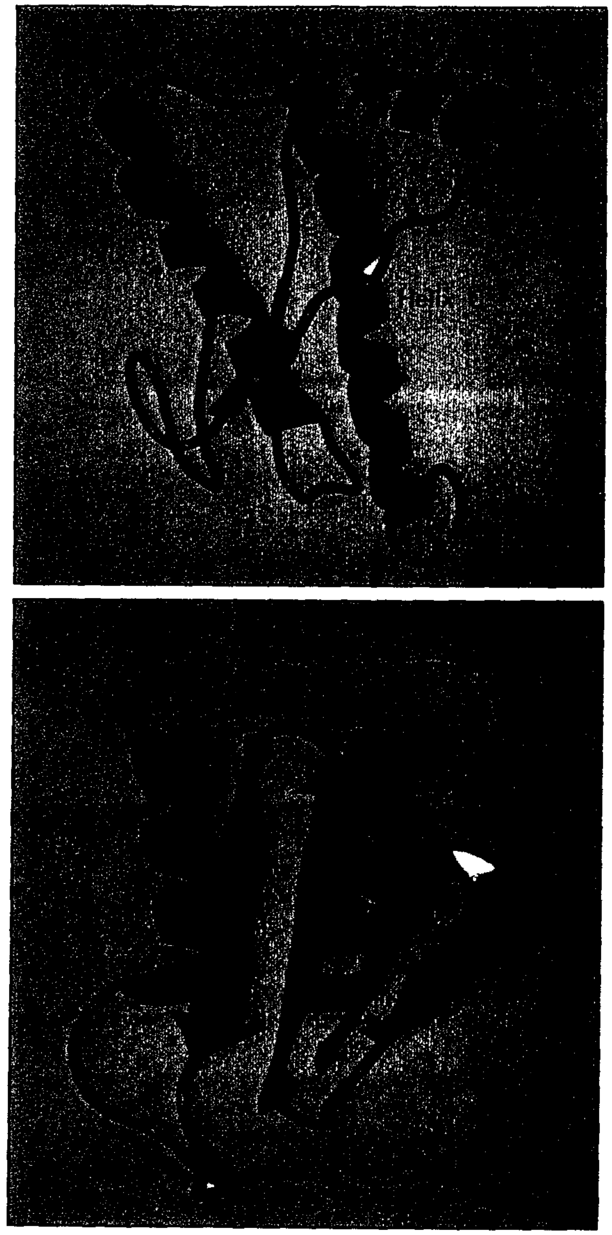

Figure 1. Structures of Prion Protein (PrP) Isoforms.

Panel A shows the α-helical structure of Syrian hamster recombinant PrP 90-231, which presumably resembles that of the cellular isoform (PrP^{C}). It is viewed from the point at which the scrapie isoform (PrP^{Sc}) is thought to bind to PrP^{C}. α-Helixes A (residues 144 through 157), B (172 through 193), and C (200 through 227) are purple, with loops in yellow; residues 129 through 134, in strand S1, and residues 159 through 165, in strand S2, are blue. Panel B shows a plausible model of the tertiary structure of human PrP^{Sc}. S1 β-strands (residues 108 through 113 and 116 through 122) and S2 β-strands (residues 128 through 135 and 138 through 144) are blue. α-Helixes B (residues 178 through 191) and C (residues 202 through 218) are purple, with yellow loops.

TABLE 2. PATHOGENETIC FEATURES OF PRION DISEASES.

DISEASE	HOST	MECHANISM OF PATHOGENESIS*
Kuru	Fore people in New Guinea	Infection through ritualistic cannibalism
Creutzfeldt–Jakob disease		
Iatrogenic	Humans	Infection from prion-contaminated human growth hormone, dura mater grafts, and so forth
New variant	Humans	Infection from bovine prions?
Familial	Humans	Germ-line mutations in the *PrP* gene
Sporadic	Humans	Somatic mutation or spontaneous conversion of PrP^{C} into PrP^{Sc}?
Gerstmann–Sträussler–Scheinker disease	Humans	Germ-line mutations in the *PrP* gene
Fatal familial insomnia	Humans	Germ-line mutations in the *PrP* gene (D178N, M129)
Sporadic fatal insomnia	Humans	Somatic mutation or spontaneous conversion of PrP^{C} into PrP^{Sc}?
Scrapie	Sheep	Infection in genetically susceptible sheep
Bovine spongiform encephalopathy	Cattle	Infection with prion-contaminated meat and bone meal
Transmissible mink encephalopathy	Mink	Infection with prions from sheep or cattle
Chronic wasting disease	Mule deer, elk	Unknown
Feline spongiform encephalopathy	Cats	Infection with prion-contaminated beef
Exotic ungulate encephalopathy	Greater kudu, nyala, oryx	Infection with prion-contaminated meat and bone meal

*A question mark indicates that the mechanism has not been confirmed. PrP denotes prion protein.

Creutzfeldt–Jakob disease is approximately 1 case per 1 million population,[23] but among persons between the ages of 60 and 74 years, the incidence is nearly 5 per 1 million.[24] Cases in patients as young as 17 years and as old as 83 have been recorded.[23,25] Creutzfeldt–Jakob disease is relentlessly progressive and usually causes death within a year after its onset. Each geographic cluster of cases of prion disease was initially thought to be a manifestation of viral communicability,[26] but each was later shown to be due to a *PRNP* gene mutation except for new variant Creutzfeldt–Jakob disease.

Neuropathological Features

There are often no recognizable gross abnormalities in the brains of patients with Creutzfeldt–Jakob disease. Patients who survive for several years have variable degrees of cerebral atrophy. The microscopical features of Creutzfeldt–Jakob disease are spongiform degeneration and astrogliosis (Fig. 2A and 2B).[27]

Amyloid plaques occur in approximately 10 percent of cases of Creutzfeldt–Jakob disease. These plaques are positive for antibodies against PrP^{Sc} on immunohistochemical staining.[28,29] The amyloid plaques in patients with Gerstmann–Sträussler–Scheinker disease consist of a dense core of amyloid surrounded by smaller globules of amyloid (Fig. 2). A characteristic feature of new variant Creutzfeldt–Jakob disease is the presence of "florid plaques" composed of a core of PrP^{Sc} amyloid surrounded by vacuoles (Fig. 2E and 2F).

Strains of Prions

The existence of prion strains raises the question of how heritable biologic information can be encrypted in a molecule other than nucleic acid.[30-32] Strains of prions have been defined by the rapidity with which they cause central nervous system disease and by the distribution of neuronal vacuolation.[30] Patterns of PrP^{Sc} deposition have also been used to characterize these strains.[33,34] There is mounting evidence that the diversity of prions is enciphered in the conformation of the PrP^{Sc} protein.[35-39] Studies involving the transmission of fatal familial insomnia and familial Creutzfeldt–Jakob disease to mice expressing a chimeric human–mouse PrP transgene have shown that the tertiary and quaternary structure of PrP^{Sc} contains strain-specific information.[37] Studies of patients with fatal sporadic insomnia have extended these findings,[40] making it clear that PrP^{Sc} acts as a template for the conversion of PrP^{C} into nascent PrP^{Sc}.

Sporadic, Genetic, and Infectious Forms of Prion Disease

Sporadic prion diseases might be initiated by a somatic mutation and in this respect might develop in a manner similar to prion diseases caused by germ-line

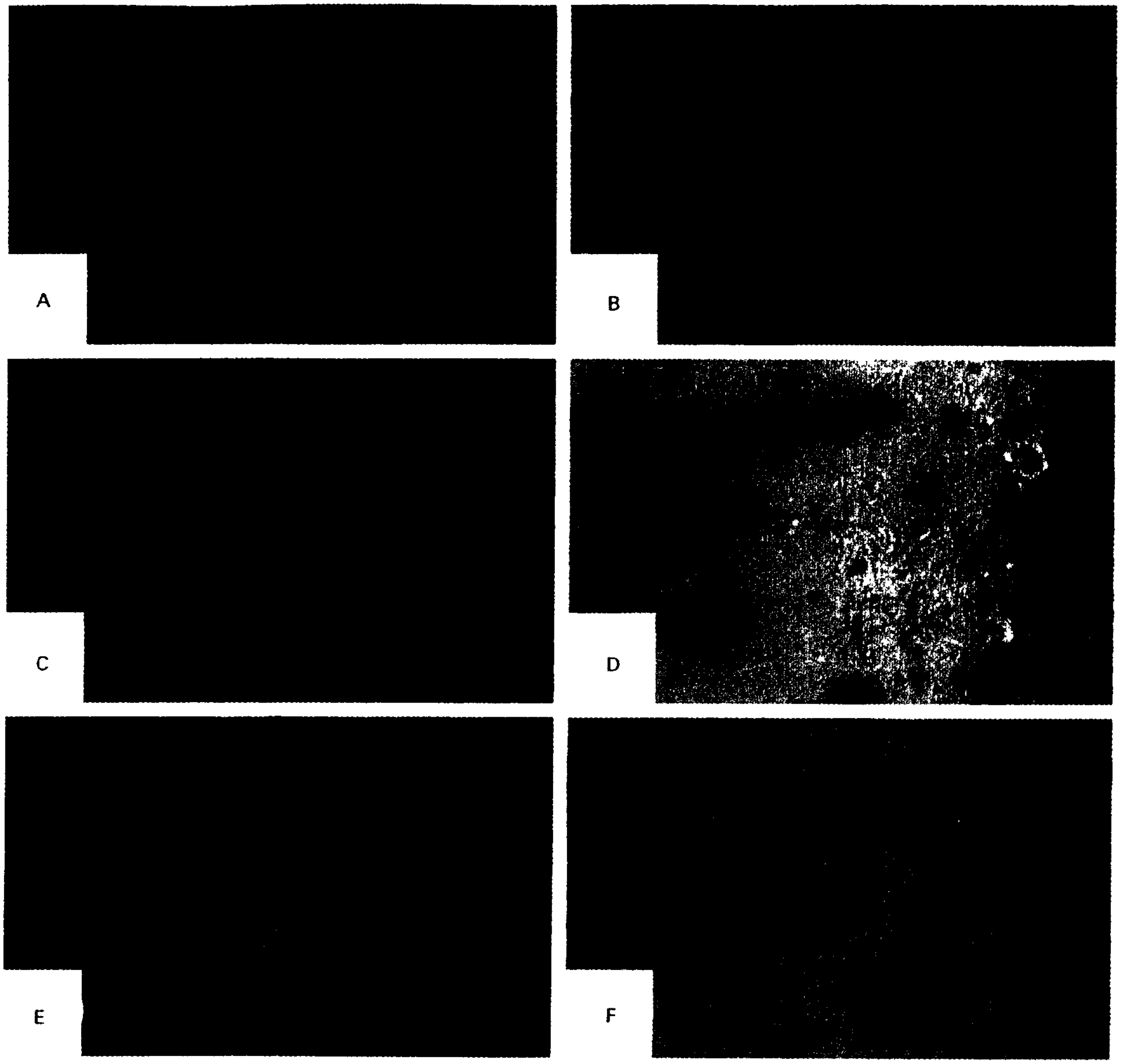

Figure 2. Neuropathological Features of Prion Diseases in Humans.

Sporadic Creutzfeldt–Jakob disease is characterized by vacuolation of the neuropil in the gray matter; by exuberant reactive astrocytic gliosis, the extent of which is proportional to the degree of nerve-cell loss; and in rare cases by the formation of prion protein (PrP) amyloid plaques. The neuropathological features of familial Creutzfeldt–Jakob disease are similar. Gerstmann–Sträussler–Scheinker disease due to a substitution at codon 102 (P102L), as well as other inherited forms of Gerstmann–Sträussler–Scheinker disease, is characterized by numerous deposits of PrP amyloid throughout the central nervous system. New variant Creutzfeldt–Jakob disease is distinguished by the abundance of PrP amyloid plaques, which are often surrounded by a halo of intense vacuolation.

Panel A shows widespread spongiform degeneration in a specimen of cerebral cortex from a patient with sporadic Creutzfeldt–Jakob disease (hematoxylin and eosin, ×200). Panel B shows widespread reactive gliosis in a specimen of cerebral cortex from a patient with sporadic Creutzfeldt–Jakob disease; the specimen is immunostained with antibodies against glial fibrillary acid protein. Panel C shows a specimen of the cerebellum from a patient with Gerstmann–Sträussler–Scheinker disease. Most of the plaques are in the molecular layer, which occupies all but the right-hand portion of the panel; many but not all of the plaques stain positively with periodic acid–Schiff (×200). Granule cells and a single Purkinje cell are present at the right-hand side of the panel. In Panel D, a specimen of the cerebellum, obtained at the same location as that in Panel C but subjected to hydrolytic autoclaving and immunostaining, reveals more PrP plaques (×100). In Panel E, a specimen of cerebral cortex obtained from a patient with new variant Creutzfeldt–Jakob disease shows amyloid deposits within vacuoles (hematoxylin and eosin, ×200). These deposits have been referred to as "florid plaques." In Panel F, a specimen of cerebral cortex obtained from the same location as that in Panel E but subjected to hydrolytic autoclaving and immunostaining for PrP reveals numerous PrP plaques, many of which are in clusters, as well as minute deposits of PrP surrounding many cortical neurons and their proximal processes (×100). The bar in Panel B represents 50 μm and also applies to Panels A, C, and E. The bar in Panel F represents 100 μm and also applies to Panel D. The specimens from the patients with new variant Creutzfeldt–Jakob disease were provided by James Ironside, Jeanne Bell, and Robert Will.

mutations. In this situation, the mutant PrP^{Sc} must be capable of recruiting wild-type PrP^{C}, a process that may occur with some mutations but is unlikely with others.[41] Alternatively, the activation barrier separating wild-type PrP^{C} from PrP^{Sc} may be crossed on rare occasions in the context of a large population of people.[42] Twenty mutations in the human *PRNP* gene have been found to segregate with inherited prion diseases.[43] Missense mutations and expansions in the octapeptide-repeat region of the gene cause familial prion diseases.[15-19]

Although infectious prion diseases constitute less than 1 percent of all cases of prion disease, the circumstances surrounding the transmission of these infectious illnesses are often dramatic (Table 2). Ritualistic cannibalism has resulted in the transmission of kuru among the Fore people of New Guinea, industrial cannibalism has been responsible for bovine spongiform encephalopathy (BSE), or "mad cow disease," in Europe, and an increasing number of patients have contracted new variant Creutzfeldt–Jakob disease from prion-tainted beef products.[13]

The restricted geographic and temporal distribution of cases of new variant Creutzfeldt–Jakob disease raises the possibility that BSE prions have been transmitted to humans. Although over 100 cases of new variant Creutzfeldt–Jakob disease have been recorded,[44,45] no dietary habits distinguish patients with this disease from apparently healthy persons. Moreover, it is unclear why teenagers and young adults seem to be particularly susceptible to the disease. These cases may mark the start of an epidemic of prion disease in Great Britain like those of BSE and kuru, or the number of cases of new variant Creutzfeldt–Jakob disease may remain small, as with iatrogenic Creutzfeldt–Jakob disease caused by cadaveric human growth hormone.[46]

The most compelling evidence that new variant Creutzfeldt–Jakob disease is caused by BSE prions comes from studies of mice expressing the bovine PrP transgene.[47] The incubation times, neuropathological features, and patterns of PrP^{Sc} deposition in these transgenic mice are the same whether the inoculate originated from the brains of cattle with BSE or from humans with new variant Creutzfeldt–Jakob disease.[47] The origin of BSE is still obscure, although epidemiologic studies indicate that BSE probably arose from a single point source in the southwest of England in the 1970s.[48] It probably originated from a rare case of prion disease in either sheep (Scott M, Prusiner SB: unpublished data) or cattle.[48] Once established, the disease was spread in cattle by ingestion of prion-contaminated meat and bone meal.

The accidental transmission of Creutzfeldt–Jakob disease to humans appears to have occurred with corneal transplantation[49] and use of contaminated electroencephalographic electrodes.[50] The same improperly decontaminated electrodes that had caused Creutzfeldt–Jakob disease in two young patients with intractable epilepsy were found to cause Creutzfeldt–Jakob disease in a chimpanzee 18 months after their implantation in the animal.[51] More than 70 cases of Creutzfeldt–Jakob disease associated with the implantation of dura mater grafts have been recorded.[52] One case occurred after the repair of a perforated eardrum with a pericardial graft.[53] Prion-contaminated human growth hormone preparations derived from human pituitary tissue have caused fatal cerebellar disorders with dementia in more than 120 patients ranging in age from 10 to 41 years.[13,54,55] Four cases of Creutzfeldt–Jakob disease have occurred in women who received human pituitary gonadotropin.[56]

Polymorphisms influence the susceptibility to sporadic, inherited, and infectious forms of prion disease. Dominant negative alleles in approximately 12 percent of the Japanese population[57] encode for lysine at position 219 and interfere with the conversion of wild-type PrP^{C} into PrP^{Sc}.[58,59] Dominant negative inhibition of prion replication has also been found in sheep, with a substitution of the basic residue arginine at position 171.[60,61]

OTHER NEURODEGENERATIVE DISEASES

Like cases of the prion diseases, most cases of Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and frontotemporal dementia are sporadic; 10 percent or less are inherited. Although age is the most important risk factor in all these sporadic forms of disease, the factors that initiate neurodegeneration remain unknown. In the prion diseases, the initial formation of PrP^{Sc} leads to an exponential increase in the protein, which can be readily transmitted to another host. In the other neurodegenerative diseases, the events that lead to the production of aberrantly processed proteins, as well as the driving forces that sustain their accumulation, are unknown. It is important to stress that in contrast to the prion diseases, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and frontotemporal dementia are not infectious and have not been transmitted to laboratory animals.

Alzheimer's Disease

$A\beta$-amyloid plaques and neurofibrillary tangles are found in both sporadic and inherited forms of Alzheimer's disease (Table 3). Like familial prion diseases, familial Alzheimer's disease has an autosomal dominant pattern of inheritance. Familial Alzheimer's disease can be caused by a mutation in the gene for amyloid precursor protein (APP), presenilin 1, or presenilin 2 (Table 4).[62] Cleavage of amyloid precursor protein at residue 671 by β-secretase and at either residue 711 or residue 713 by γ-secretase produces $A\beta(1-40)$ and $A\beta(1-42)$, respectively. $A\beta(1-42)$ forms amyloid fibrils readily and is thought to cause central nervous system dysfunction before it is deposited in plaques.[63-65] Presenilin 1 and presenilin 2 may form

TABLE 3. PROTEIN DEPOSITION IN NEURODEGENERATIVE DISEASES.*

DISEASE	PROTEIN	PATHOLOGICAL FINDING
Prion diseases	PrP^{Sc}	PrP amyloid plaques
Alzheimer's disease	Aβ	Aβ amyloid plaques
	Tau	Paired helical filaments in neurofibrillary tangles
Parkinson's disease	α-Synuclein	Lewy bodies
Frontotemporal dementia	Tau	Straight filaments and paired helical filaments
Pick's disease	Tau	Pick bodies
Progressive supranuclear palsy	Tau	Straight filaments in neurofibrillary tangles
Amyotrophic lateral sclerosis	Neurofilament	Neuronal aggregates
Huntington's disease	Huntingtin	Nuclear inclusions
Spinocerebellar ataxia		
Type 1	Ataxin 1	Nuclear inclusions
Type 2	Ataxin 2	Cytoplasmic inclusions
Machado–Joseph disease	Ataxin 3	Nuclear inclusions

*PrP denotes prion protein, and PrP^{Sc} the scrapie isoform of PrP.

complexes with at least one other protein, nicastrin, a transmembrane neuronal glycoprotein, and these complexes may contribute to the production of Aβ(1–42).[66]

The age of onset of both sporadic and familial forms of Alzheimer's disease is modulated by allelic variants of apolipoprotein E.[67] Three alternative allelic products of apolipoprotein E, denoted ε2, ε3, and ε4, differ at amino acid residues 112 and 158. In many persons with two ε4 alleles, Alzheimer's disease develops at least a decade before it does in those with two copies of ε2, and ε3 is associated with an onset of disease at an intermediate age.[68]

Frontotemporal Dementia and Pick's Disease

Mutations in the *tau* gene, which codes for tau, a protein associated with microtubules, cause inherited forms of frontotemporal dementia and Pick's disease.[69-71] As with Alzheimer's disease, about 90 percent of cases of frontotemporal dementia are sporadic, and the rest are familial. Straight filaments composed of hyperphosphorylated mutant tau have been found in the brains of patients with familial frontotemporal dementia (Table 3).[72] In some cases, neurofibrillary tangles composed of paired helical filaments have been found; the formation of these filaments seems to depend on the specific mutation and on the specific isoform of the protein (Table 4).[73] In sporadic cases of frontotemporal dementia, aggregates of tau are uncommon. Approximately 15 percent of patients with frontotemporal dementia have Pick bodies,[74] which are intracellular collections of partially degraded (ubiquinated) tau fibrils in the brain.[75] As with frontotemporal dementia, most cases of Pick's disease are sporadic. Other disorders caused by the misprocessing of tau include progressive supranuclear palsy, progressive subcortical gliosis, and corticobasal degeneration.[73,75-77]

TABLE 4. MUTANT GENES IN FAMILIAL NEURODEGENERATIVE DISEASES.

DISEASE	GENE	MUTATION
Prion diseases	*PRNP*	Point mutations and octapeptide repeats
Alzheimer's disease	*APP*	Point mutations
	PS1	Point mutations
	PS2	Point mutations
Parkinson's disease	*SNCA*	Point mutations
	parkin	Point mutations
Frontotemporal dementia	*tau*	Point mutations, deletions
Pick's disease	*tau*	Point mutations
Amyotrophic lateral sclerosis	*SOD1*	Point mutations
Huntington's disease	*HD*	Polyglutamine expansions
Spinocerebellar ataxia		
Type 1	*SCA1*	Polyglutamine expansions
Type 2	*SCA2*	Polyglutamine expansions
Machado–Joseph disease	*SCA3*	Polyglutamine expansions

Parkinson's Disease

Most cases of Parkinson's disease are sporadic,[78,79] but both sporadic and familial forms of the disease are characterized by protein deposits in the central nervous system. Mutations in the gene for α-synuclein have been found in patients with familial Parkinson's disease.[80] In both sporadic and familial cases, antibodies to α-synuclein, a presynaptic intracellular protein, stain Lewy bodies in neurons of the substantia nigra.[81] Whereas the inheritance of Parkinson's disease due to mutations in the α-synuclein gene is autosomal dominant, a childhood form of the disease due to mutations in the gene for ubiquitin–protein ligase (*parkin*) is a recessive disorder (Table 4).[82] Parkin seems to promote the degradation of certain neuronal proteins, and selective nitration of α-synuclein has been observed in Lewy bodies.[83]

Parkinson's disease in older persons is associated with a high incidence of dementia.[84] At autopsy, the brains of such patients often have the neuropathological hallmarks of both Alzheimer's disease and Parkinson's disease. Immunohistochemical studies showing the presence of α-synuclein in cortical Lewy bodies have helped resolve the conundrum of how a patient could have insufficient numbers of plaques and neurofibrillary tangles for the diagnosis of Alzheimer's disease but still have dementia. The presence of these α-synuclein deposits, alone or in combination with changes that are characteristic of Alzheimer's disease, may be the second most common form of neurodegeneration, accounting for 20 to 30 percent of cases of dementia among persons over the age of 60 years.[85,86] A small number of younger persons with Parkinson's disease also have dementia due to diffuse Lewy body disease.[87]

Amyotrophic Lateral Sclerosis

Although most cases of amyotrophic lateral sclerosis are sporadic, familial cases have been identified.[88-90] In approximately 20 percent of familial cases of amyotrophic lateral sclerosis, there are mutations in the gene for cytoplasmic superoxide dismutase type 1 (SOD1) (Table 4).[91] Moreover, deposits of SOD1 in the central nervous system have been found in both sporadic and familial cases of amyotrophic lateral sclerosis.[92] Although in some cases abnormal collections of neurofilaments have been seen in degenerating motor neurons, no familial cases have been shown to be due to mutations in neurofilament genes.[92]

Huntington's Disease and Spinocerebellar Ataxias

Unlike Alzheimer's disease, frontotemporal dementia, Parkinson's disease, amyotrophic lateral sclerosis, and the prion diseases, which in most cases are sporadic, all cases of Huntington's disease and of spinocerebellar ataxia are caused by expanded polyglutamine repeats (Table 4).[93-95] But these diseases are similar to the inherited forms of Alzheimer's disease, frontotemporal dementia, Parkinson's disease, amyotrophic lateral sclerosis, and the prion diseases in that they are usually manifested as neurologic deficits in adulthood, even though the expression of the mutant gene products in the central nervous system begins early in life. Childhood forms of Huntington's disease and spinocerebellar ataxia are known to be due to large expansions of the causative triplet repeats.[94,96,97]

Transgenic Mouse Models

Although virtually every facet of the human and animal prion diseases has been reproduced in transgenic mice, attempts to develop transgenic models for the other neurodegenerative diseases have proved more difficult. Despite the lack of perfect transgenic models for Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, frontotemporal dementias, Huntington's disease, and the spinocerebellar ataxias, many aspects of these human disorders have been reproduced. Mice expressing transgenes carrying mutations found in the inherited forms of these neurodegenerative diseases develop disorders with many of the neuropathological features that characterize the corresponding human illnesses (Tables 3 and 4).

DIAGNOSTIC TESTS

There is an urgent need for a rapid, antemortem test for prions in humans and livestock. A highly sensitive quantitative immunoassay has been developed on the basis of antigens that are exposed in PrP^{C} but buried in PrP^{Sc}. Unlike earlier immunoassays for PrP^{Sc}, this conformation-dependent immunoassay does not require limited proteolysis to hydrolyze PrP^{C} before the protease-resistant core of PrP^{Sc} (PrP 27–30) is measured.[38] This assay has been used to identify a new form of PrP^{Sc}, which is protease-sensitive ($sPrP^{Sc}$).

A diagnostic test would be valuable for distinguishing between early Alzheimer's disease and depression in older persons, since both disorders are so common. In Alzheimer's disease, frontotemporal dementia, Parkinson's disease, and the prion diseases, computed tomography or magnetic resonance imaging may show normal findings or cortical atrophy. In patients with Alzheimer's disease, widespread atrophy with enlarged ventricles is often seen, especially late in the disease, but this finding is not diagnostic. Many elderly persons with normal cognition have similar radiographic findings.[98,99] Although many patients with Creutzfeldt–Jakob disease have elevated levels of protein 14-3-3 in cerebrospinal fluid, this finding is not specific for the diagnosis.[100,101] Attempts to measure $A\beta(1-40)$ in blood and urine as diagnostic tests have been unrewarding,[102] but the use of fluorescence correlation spectroscopy to measure $A\beta(1-40)$ in cerebrospinal fluid may provide a reliable diagnostic test for Alzheimer's disease.[103]

Whereas electroencephalographic studies are not useful for the diagnosis of Alzheimer's disease, frontotemporal dementia, or Parkinson's disease, they are often useful for the diagnosis of Creutzfeldt–Jakob disease. Repetitive, high-voltage, triphasic and polyphasic sharp discharges are seen in most advanced cases of Creutzfeldt–Jakob disease, but their presence is often transient.[25,101,104,105] As the disease progresses, normal background rhythms become fragmentary and slower.

Hashimoto's thyroiditis should always be considered in the differential diagnosis of Creutzfeldt–Jakob disease,[106] since the former disorder is a treatable autoimmune disease whereas Creutzfeldt–Jakob disease is not. The clinical and neuropathological findings in these two disorders can be quite similar, raising the possibility that protein misprocessing underlies both degenerative and autoimmune diseases.

PREVENTION AND TREATMENT

With the exception of levodopa, which ameliorates the symptoms of Parkinson's disease but does not halt the underlying degeneration, there are no effective therapies for neurodegenerative diseases. The history of successful attempts to prevent or reverse protein misprocessing is extremely limited.[107] Developing new drugs directed to specific regions of the central nervous system will be challenging.

Preventing Abnormal Processing of Proteins and Enhancing Their Clearance

Structure-based drug design based on dominant negative inhibition of prion formation has resulted in the development of several compounds.[108] However, the task of exchanging polypeptide scaffolds for small heterocyclic structures without the loss of biologic activity remains difficult. Whether this approach to preventing the aberrant processing of proteins will lead to the development of new treatments for Alzheimer's and Parkinson's diseases, as well as other neurodegenerative disorders, remains to be established.

Several compounds can eliminate prions from cultured cells. A class of compounds known as "dendrimers" seems particularly effective in this regard.[109] Some drugs delay the onset of disease in animals that have been inoculated with prions if the drugs are given around the time of the inoculation.[110] A novel approach to treating Alzheimer's disease has been developed in transgenic mice that overexpress a mutant *APP* gene. Immunization of these mice with the Aβ peptide or injection of antibodies to Aβ reduces plaque formation.[111] Whether this approach will prove fruitful in patients is unknown.

Replacement Therapy

Because the neurodegeneration in Parkinson's disease is confined largely to the substantia nigra, especially early in the disease process, replacement therapy with levodopa has proved useful; in many patients, however, the disease eventually becomes refractory to levodopa.[112] Similar approaches to the treatment of Alzheimer's disease have been disappointing, primarily because the disease process is so widespread. Similarly, the widespread neuropathological changes in amyotrophic lateral sclerosis, frontotemporal dementia, and prion diseases make it unlikely that replacement therapy will be successful.

SPECULATION ON THE SPECTRUM OF DEGENERATIVE DISEASES

It is tempting to speculate that abnormal processing of neuronal proteins also occurs in other diseases of the central nervous system, such as schizophrenia, bipolar disorders, autism, and narcolepsy.[113] Most cases of these diseases are sporadic, but a substantial minority appear to be familial. The absence of neuropathological changes in these conditions has impeded phenotypic analysis. In a group of patients with inherited frontotemporal dementia who have a mutation in the *tau* gene, alcoholism and Parkinson's disease are prominent features.[114]

Whether multiple sclerosis is also the result of defective processing of brain proteins is unknown.[115] The immune system features prominently in the pathogenesis of multiple sclerosis, and it is often argued that this disease is a T-cell–mediated, autoimmune disorder. Antibody-mediated demyelination has been found in some cases of multiple sclerosis,[116] and in others, degeneration of oligodendrocytes has been observed, with little or no evidence of immune-mediated damage.[117] Perhaps ulcerative colitis, Crohn's disease, rheumatoid arthritis, type 1 diabetes mellitus, and systemic lupus erythematosus ought to be considered disorders of protein processing in which misfolded proteins evoke an autoimmune response.

The systemic amyloidoses share important features with the neurodegenerative diseases. In primary amyloidosis, immunoglobulin light chains form amyloid deposits that can cause cardiomyopathy, renal failure, and polyneuropathy.[118] In response to chronic inflammatory diseases, the serum amyloid A protein is cleaved and forms the amyloid A protein, which is deposited as fibrils in the kidney, liver, and spleen. The most common form of systemic hereditary amyloidosis is caused by the deposition of mutant transthyretin. Also noteworthy are amylin deposits in the β-islet cells of patients with type 2 diabetes mellitus. These deposits contain amyloid fibrils that are composed of the amylin protein.

THE FUTURE

As life expectancy continues to increase, the burden of degenerative diseases is growing. Developing effective means of preventing these disorders and of treating them when they do occur is a paramount challenge. The problems caused by Alzheimer's disease and Parkinson's disease are already so great that if the prevalence of these maladies continues to increase in accordance with the changing demographic characteristics of the world population, they will bankrupt both developed and developing countries over the next 50 years. It is remarkable to think that by the year 2025, more than 65 percent of persons over the age of 65 years will be living in countries that are now designated as developing countries.[119] Unless effective methods of prevention and treatment are developed, this enormous population of people will be subjected to the same risks of Alzheimer's disease, Parkinson's disease, and other neurodegenerative disorders as are older persons currently living in the most affluent countries.

Over the past two decades, remarkable progress has been made in elucidating the causes of neurodegenerative diseases, and the time has come to intensify the search for drug targets and for compounds that interrupt the disease processes. Drugs that block the mishandling of a particular protein may be most effective for certain disorders; for others, drugs that enhance the clearance of an aberrant protein or fragment may prove most useful. Regardless of the therapeutic approach, accurate, early detection of neurodegeneration will be extremely important so that drugs can be given before substantial damage to the central nervous system has occurred. However, the enormity of these tasks — developing useful diagnostic tests and discovering effective therapies — should not be underestimated.

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REFERENCES

1. Prusiner SB. Development of the prion concept. In: Prusiner SB, ed. Prion biology and diseases. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press, 1999:67-112.
2. *Idem.* Some speculations about prions, amyloid, and Alzheimer's disease. N Engl J Med 1984;310:661-3.
3. Lilienfeld DE. An epidemiological overview of amyotrophic lateral sclerosis, Parkinson's disease, and dementia of the Alzheimer type. In: Calne DB, ed. Neurodegenerative diseases. Philadelphia: W.B. Saunders, 1994:399-425.
4. Kawas CH, Katzman R. Epidemiology of dementia and Alzheimer disease. In: Terry RD, Katzman R, Bick KL, Sisodia SS, eds. Alzheimer disease. 2nd ed. Philadelphia: Lippincott Williams & Wilkins, 1999:95-116.
5. Tanner CM, Goldman SM. Epidemiology of Parkinson's disease. Neuroepidemiology 1996;14:317-35.
6. Evans DA, Funkenstein HH, Albert MS, et al. Prevalence of Alzheimer's disease in a community population of older persons: higher than previously reported. JAMA 1989;10:2551-6.
7. Bennett DA, Beckett LA, Murray AM, et al. Prevalence of parkinsonian signs and associated mortality in a community population of older people. N Engl J Med 1996;334:71-6.
8. Prusiner SB. Prions. Proc Natl Acad Sci U S A 1998;95:13363-83.
9. McKinley MP, Meyer RK, Kenaga L, et al. Scrapie prion rod formation *in vitro* requires both detergent extraction and limited proteolysis. J Virol 1991;65:1340-51.
10. Pan K-M, Baldwin M, Nguyen J, et al. Conversion of α-helices into β-sheets features in the formation of the scrapie prion proteins. Proc Natl Acad Sci U S A 1993;90:10962-6.
11. Riek R, Hornemann S, Wider G, Billeter M, Glockshuber R, Wüthrich K. NMR structure of the mouse prion protein domain PrP(121-231). Nature 1996;382.180-2.
12. Liu H, Farr-Jones S, Ulyanov NB, et al. Solution structure of Syrian hamster prion protein rPrP(90-231). Biochemistry 1999;38:5362-77.
13. Will RG, Alpers MP, Dormont D, Schonberger LB, Tateishi J. Infectious and sporadic prion diseases. In: Prusiner SB, ed. Prion biology and diseases. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press, 1999:465-507.
14. DeArmond SJ, Prusiner SB. Prion diseases. In: Graham DI, Lantos PL, eds. Greenfield's neuropathology. 6th ed. London: Arnold, 1997:235-80.
15. Hsiao K, Baker HF, Crow TJ, et al. Linkage of a prion protein missense variant to Gerstmann-Sträussler syndrome. Nature 1989;338:342-5
16. Dlouhy SR, Hsiao K, Farlow MR, et al. Linkage of the Indiana kindred of Gerstmann-Sträussler-Scheinker disease to the prion protein gene. Nat Genet 1992;1:64-7
17. Petersen RB, Tabaton M, Berg L, et al. Analysis of the prion protein gene in thalamic dementia. Neurology 1992;42:1859-63.
18. Poulter M, Baker HF, Frith CD, et al. Inherited prion disease with 144 base pair gene insertion. 1. Genealogical and molecular studies. Brain 1992;115:675-85.
19. Gabizon R, Rosenmann H, Meiner Z, et al. Mutation and polymorphism of the prion protein gene in Libyan Jews with Creutzfeldt-Jakob disease (CJD). Am J Hum Genet 1993;53:828-35.
20. Roos R, Gajdusek DC, Gibbs CJ Jr. The clinical characteristics of transmissible Creutzfeldt-Jakob disease. Brain 1973;96:1-20.
21. Masters CL, Gajdusek DC, Gibbs CJ Jr. Creutzfeldt-Jakob disease virus isolations from the Gerstmann-Sträussler syndrome with an analysis of the various forms of amyloid plaque deposition in the virus-induced spongiform encephalopathies. Brain 1981;104.559-88.
22. Hsiao K, Doh-ura K, Kitamoto T, Tateishi J, Prusiner SB. A prion protein amino acid substitution in ataxic Gerstmann-Sträussler syndrome. Ann Neurol 1989;26:137. abstract.
23. Masters CL, Harris JO, Gajdusek DC, Gibbs CJ, Bernoulli C, Asher DM. Creutzfeldt-Jakob disease: patterns of worldwide occurrence and the significance of familial and sporadic clustering. Ann Neurol 1979;5:177-88.
24. Holman RC, Khan AS, Belay ED, Schonberger LB. Creutzfeldt-Jakob disease in the United States, 1979-1994: using national mortality data to assess the possible occurrence of variant cases. Emerg Infect Dis 1996;2:333-7.
25. Cathala F, Baron H. Clinical aspects of Creutzfeldt-Jakob disease. In: Prusiner SB, McKinley MP, eds. Prions — novel infectious pathogens causing scrapie and Creutzfeldt-Jakob disease. Orlando, Fla.: Academic Press, 1987:467-509.
26. Kahana E, Alter M, Braham J, Sofer D. Creutzfeldt-Jakob disease: focus among Libyan Jews in Israel. Science 1974;183:90-1.
27. DeArmond SJ, Ironside JW. Neuropathology of prion diseases. In: Prusiner SB, ed. Prion biology and diseases. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press, 1999:585-652.
28. Bendheim PE, Barry RA, DeArmond SJ, Stites DP, Prusiner SB. Antibodies to a scrapie prion protein. Nature 1984;310:418-21.
29. DeArmond SJ, McKinley MP, Barry RA, Braunfeld MB, McColloch JR, Prusiner SB. Identification of prion amyloid filaments in scrapie-infected brain. Cell 1985,41:221-35.
30. Dickinson AG, Meikle VMH, Fraser H. Identification of a gene which controls the incubation period of some strains of scrapie agent in mice. J Comp Pathol 1968;78:293-9.
31. Bruce ME, Dickinson AG. Biological evidence that scrapie agent has an independent genome. J Gen Virol 1987;68:79-89.
32. Ridley RM, Baker HF. To what extent is strain variation evidence for an independent genome in the agent of the transmissible spongiform encephalopathies? Neurodegeneration 1996;5:219-31.
33. DeArmond SJ, Mobley WC, DeMott DL, Barry RA, Beckstead JH, Prusiner SB. Changes in the localization of brain prion proteins during scrapie infection. Neurology 1987;37:1271-80 [Erratum, Neurology 1987;37:1770.]
34. Bruce ME, McBride PA, Farquhar CF. Precise targeting of the pathology of the sialoglycoprotein, PrP, and vacuolar degeneration in mouse scrapie. Neurosci Lett 1989;102:1-6.
35. Prusiner SB. Molecular biology of prion diseases. Science 1991;252:1515-22.
36. Bessen RA, Marsh RF. Distinct PrP properties suggest the molecular basis of strain variation in transmissible mink encephalopathy. J Virol 1994;68:7859-68
37. Telling GC, Parchi P, DeArmond SJ, et al. Evidence for the conformation of the pathologic isoform of the prion protein enciphering and propagating prion diversity. Science 1996;274:2079-82.
38. Scott MR, Groth D, Tatzelt J, et al. Propagation of prion strains through specific conformers of the prion protein. J Virol 1997;71:9032-44.
39. Safar J, Wille H, Itri V, et al. Eight prion strains have PrP^{Sc} molecules with different conformations. Nat Med 1998;4:1157-65.
40. Mastrianni JA, Nixon R, Layzer R, et al. Prion protein conformation in a patient with sporadic fatal insomnia. N Engl J Med 1999;340:1630-8.
41. Telling GC, Scott M, Mastrianni J, et al. Prion propagation in mice expressing human and chimeric PrP transgenes implicates the interaction of cellular PrP with another protein. Cell 1995;83:79-90.
42. Cohen FE, Prusiner SB. Pathologic conformations of prion proteins. Annu Rev Biochem 1998;67:793-819.
43. Gambetti P, Petersen RB, Parchi P, et al. Inherited prion diseases. In: Prusiner SB, ed. Prion biology and diseases. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press, 1999:509-83.
44. Will RG, Cousens SN, Farrington CP, Smith PG, Knight RSG, Ironside JW. Deaths from variant Creutzfeldt-Jakob disease. Lancet 1999;353:979.
45. Balter M. Tracking the human fallout from 'mad cow disease.' Science 2000,289 1452-4.
46. Ghani AC, Ferguson NM, Donnelly CA, Anderson RM. Predicted vCJD mortality in Great Britain. Nature 2000,406:583-4.
47. Scott MR, Will R, Ironside J, et al. Compelling transgenetic evidence for transmission of bovine spongiform encephalopathy prions to humans. Proc Natl Acad Sci U S A 1999,96 15137-42

48. Phillips N, Bridgeman J, Ferguson-Smith M. BSE inquiry report. Vol 2. Science. London: Stationery Office, 2000.
49. Duffy P, Wolf J, Collins G, DeVoe AG, Streeten B, Cowen D. Possible person-to-person transmission of Creutzfeldt–Jakob disease. N Engl J Med 1974;290:692-3.
50. Bernoulli C, Siegfried J, Baumgartner G, et al. Danger of accidental person-to-person transmission of Creutzfeldt-Jakob disease by surgery. Lancet 1977;1:478-9.
51. Gibbs CJ, Asher DM, Kobrine A, Amyx HL, Sulima MP, Gajdusek DC. Transmission of Creutzfeldt-Jakob disease to a chimpanzee by electrodes contaminated during neurosurgery. J Neurol Neurosurg Psychiatry 1994;57:757-8.
52. Creutzfeldt-Jakob disease associated with cadaveric dura mater grafts — Japan, January 1979–May 1996. MMWR Morb Mortal Wkly Rep 1997; 46:1066-9.
53. Tange RA, Troost D, Limburg M. Progressive fatal dementia (Creutzfeldt-Jakob disease) in a patient who received homograft tissue for tympanic membrane closure. Eur Arch Otorhinolaryngol 1989;247:199-201
54. Fradkin JE, Schonberger LB, Mills JL, et al. Creutzfeldt-Jakob disease in pituitary growth hormone recipients in the United States. JAMA 1991; 265:880-4.
55. Report on human growth hormone and Creutzfeldt-Jakob disease. Vol. 14. Washington, D.C.: Public Health Service Interagency Coordinating Committee, 1997:1-11.
56. Cochius JI, Burns RJ, Blumbergs PC, Mack K, Alderman CP. Creutzfeldt-Jakob disease in a recipient of human pituitary-derived gonadotrophin. Aust N Z J Med 1990;20:592-3.
57. Shibuya S, Higuchi J, Shin R-W, Tateishi J, Kitamoto T. Codon 219 Lys allele of PRNP is not found in sporadic Creutzfeldt-Jakob disease. Ann Neurol 1998;43:826-8.
58. Kaneko K, Zulianello L, Scott M, et al. Evidence for protein X binding to a discontinuous epitope on the cellular prion protein during scrapie prion propagation. Proc Natl Acad Sci U S A 1997;94:10069-74.
59. Zulianello L, Kaneko K, Scott M, et al. Dominant-negative inhibition of prion formation diminished by deletion mutagenesis of the prion protein. J Virol 2000;74:4351-60.
60. Westaway D, Zuliani V, Cooper CM, et al. Homozygosity for prion protein alleles encoding glutamine-171 renders sheep susceptible to natural scrapie. Genes Dev 1994;8:959-69.
61. Hunter N, Moore L, Hosie BD, Dingwall WS, Greig A. Association between natural scrapie and PrP genotype in a flock of Suffolk sheep in Scotland. Vet Rec 1997;140:59-63.
62. St George-Hyslop PH. Molecular genetics of Alzheimer disease. In: Terry RD, Katzman R, Bick KL, Sisodia SS, eds. Alzheimer disease. 2nd ed. Philadelphia: Lippincott Williams & Wilkins, 1999:311-26.
63. Wilson CA, Doms RW, Lee VM-Y. Intracellular APP processing and Aβ production in Alzheimer disease. J Neuropathol Exp Neurol 1999;58: 787-94.
64. Selkoe DJ Translating cell biology into therapeutic advances in Alzheimer's disease. Nature 1999;399:Suppl:A23-A31.
65. De Strooper B, Annaert W. Proteolytic processing and cell biological functions of the amyloid precursor protein. J Cell Sci 2000;113:1857-70.
66. Yu G, Nishimura M, Arawaka S, et al. Nicastrin modulates presenilin-mediated *notch/glp-1* signal transduction and βAPP processing. Nature 2000;407:48-54.
67. Saunders AM, Strittmatter WJ, Schmechel D, et al. Association of apolipoprotein E allele ε4 with late-onset familial and sporadic Alzheimer's disease. Neurology 1993;43:1467-72.
68. Farrer LA, Cupples LA, Haines JL, et al. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease: a meta-analysis. JAMA 1997;278:1349-56.
69. Clark LN, Poorkaj P, Wszolek Z, et al. Pathogenic implications of mutations in the tau gene in pallido-ponto-nigral degeneration and related neurodegenerative disorders linked to chromosome 17. Proc Natl Acad Sci U S A 1998;95:13103-7.
70. Hutton M, Lendon CL, Rizzu P, et al. Association of missense and 5′-splice-site mutations in tau with the inherited dementia FTDP-17. Nature 1998;393:702-5.
71. Spillantini MG, Murrell JR, Goedert M, Farlow MR, Klug A, Ghetti B. Mutation in the tau gene in familial multiple system tauopathy with presenile dementia. Proc Natl Acad Sci U S A 1998;95:7737-41.
72. Hong M, Zhukareva V, Vogelsberg-Ragaglia V, et al. Mutation-specific functional impairments in distinct tau isoforms of hereditary FTDP-17. Science 1998;282:1914-7.
73. Buée L, Bussière T, Buée-Scherrer V, Delacourte A, Hof PR. Tau protein isoforms, phosphorylation and role in neurodegenerative disorders. Brain Res Brain Res Rev 2000;33:95-130.
74. Brun A. Frontal lobe degeneration of non-Alzheimer type revisited. Dementia 1993,4:126-31
75. Kertesz A, Munoz DG. Pick's disease and Pick complex. New York: Wiley–Liss, 1998:301.
76. Conrad C, Andreadis A, Trojanowski JQ, et al Genetic evidence for the involvement of tau in progressive supranuclear palsy Ann Neurol 1997; 41:277-81.
77. Goedert M, Spillantini MG, Crowther RA, et al. *Tau* gene mutation in familial progressive subcortical gliosis. Nat Med 1999;5:454-7.
78. Nussbaum RL, Polymeropoulos MH Genetics of Parkinson's disease. Hum Mol Genet 1997,6:1687-91.
79. Tanner CM, Ottman R, Goldman SM, et al. Parkinson disease in twins: an etiologic study. JAMA 1999;281:341-6.
80. Polymeropoulos MH, Lavedan C, Leroy E, et al. Mutation in the α-synuclein gene identified in families with Parkinson's disease. Science 1997;276.2045-7.
81. Spillantini MG, Schmidt ML, Lee VM-Y, Trojanowski JQ, Jakes R, Goedert M. α-Synuclein in Lewy bodies. Nature 1997;388:839-40.
82. Shimura H, Hattori N, Kubo S-I, et al. Familial Parkinson disease gene product, parkin, is a ubiquitin-protein ligase. Nat Genet 2000;25: 302-5.
83. Giasson BI, Duda JE, Murray IVJ, et al. Oxidative damage linked to neurodegeneration by selective α-synuclein nitration in synucleinopathy lesions. Science 2000;290:985-9.
84. Hughes TA, Ross HF, Musa S, et al. A 10-year study of the incidence of and factors predicting dementia in Parkinson's disease. Neurology 2000; 54:1596-602.
85. Hansen L, Salmon D, Galasko D, et al. The Lewy body variant of Alzheimer's disease: a clinical and pathologic entity. Neurology 1990;40:1-8.
86. Hashimoto M, Masliah E. α-Synuclein in Lewy body disease and Alzheimer's disease Brain Pathol 1999;9:707-20.
87. Spillantini MG, Crowther RA, Jakes R, Hasegawa M, Goedert M. α-Synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with Lewy bodies. Proc Natl Acad Sci U S A 1998;95: 6469-73
88. Hudson AJ. Amyotrophic lateral sclerosis and its association with dementia, parkinsonism and other neurological disorders: a review. Brain 1981;104:217-47.
89. Swash M. Clinical features and diagnosis of amyotrophic lateral sclerosis. In: Brown RH Jr, Meininger V, Swash M, eds. Amyotrophic lateral sclerosis. London: Martin Dunitz, 2000:3-30.
90. Bobowick AR, Brody JA. Epidemiology of motor-neuron diseases. N Engl J Med 1973;288:1047-55.
91. Rosen DR, Siddique T, Patterson D, et al. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature 1993;362:59-62. [Erratum, Nature 1993;364:362.]
92. Cleveland DW, Liu J. Oxidation versus aggregation — how do SOD1 mutants cause ALS? Nat Med 2000;6:1320-1.
93. Martin JB. Molecular basis of the neurodegenerative disorders. N Engl J Med 1999;340:1970-80. [Erratum, N Engl J Med 1999;341:1407.]
94. Lin X, Cummings CJ, Zoghbi HY. Expanding our understanding of polyglutamine diseases through mouse models. Neuron 1999;24:499-502.
95. Paulson HL. Protein fate in neurodegenerative proteinopathies: polyglutamine diseases join the (mis)fold. Am J Hum Genet 1999;64:339-45.
96. The Huntington's Disease Collaborative Research Group. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell 1993;72:971-83.
97. Zoghbi HY, Orr HT. Glutamine repeats and neurodegeneration. Annu Rev Neurosci 2000;23:217-47.
98. Gertz HJ, Henkes H, Cervos-Navarro J. Creutzfeldt-Jakob disease: correlation of MRI and neuropathologic findings. Neurology 1988,38: 1481-2.
99. Kitagaki H, Mori E, Yamaji S, et al. Frontotemporal dementia and Alzheimer disease: evaluation of cortical atrophy with automated hemispheric surface display generated with MR images. Radiology 1998;208: 431-9.
100. Zerr I, Bodemer M, Gefeller O, et al. Detection of 14-3-3 protein in the cerebrospinal fluid supports the diagnosis of Creutzfeldt-Jakob disease. Ann Neurol 1998;43 32-40.
101. Johnson RT, Gibbs CJ Jr. Creutzfeldt–Jakob disease and related transmissible spongiform encephalopathies. N Engl J Med 1998;339: 1994-2004.
102. Ghiso J, Calero M, Matsubara E, et al. Alzheimer's soluble amyloid β is a normal component of human urine. FEBS Lett 1997;408:105-8.
103. Pitschke M, Prior R, Haupt M, Riesner D. Detection of single amyloid beta-protein aggregates in the cerebrospinal fluid of Alzheimer's patients by fluorescence correlation spectroscopy. Nat Med 1998;4:832-4.
104. Kirschbaum WR. Jakob-Creutzfeldt disease. Amsterdam: Elsevier, 1968:251.

105. Nevin S, McMenemey WH, Behrman S, Jones DP. Subacute spongiform encephalopathy — a subacute form of encephalopathy attributable to vascular dysfunction (spongiform cerebral atrophy). Brain 1960;83:519-64.
106. Seipelt M, Zerr I, Nau R, et al. Hashimoto's encephalitis as a differential diagnosis of Creutzfeldt-Jakob disease. J Neurol Neurosurg Psychiatry 1999;66:172-6.
107. Orr HT, Zoghbi HY. Reversing neurodegeneration: a promise unfolds. Cell 2000;101:1-4.
108. Perrier V, Wallace AC, Kaneko K, Safar J, Prusiner SB, Cohen FE Mimicking dominant negative inhibition of prion replication through structure-based drug design. Proc Natl Acad Sci U S A 2000,97:6073-8.
109. Supattapone S, Nguyen H-OB, Cohen FE, Prusiner SB, Scott MR. Elimination of prions by branched polyamines and implications for therapeutics. Proc Natl Acad Sci U S A 1999;96:14529-34.
110. Priola SA, Raines A, Caughey WS. Porphyrin and phthalocyanine antiscrapie compounds. Science 2000;287:1503-6.
111. Schenk D, Barbour R, Dunn W, et al. Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. Nature 1999;400:173-7.
112. Marsden CD, Parkes JD. Success and problems of long-term levodopa therapy in Parkinson's disease. Lancet 1977;1:345-9.
113. van den Pol AN. Narcolepsy: a neurodegenerative disease of the hypocretin system? Neuron 2000;27:415-8.
114. Wilhelmsen KC, Lynch T, Pavlou E, Higgins M, Nygaard TG. Localization of disinhibition-dementia-parkinsonism-amyotrophy complex to 17q21-22. Am J Hum Genet 1994;55:1159-65.
115. Seboun E, Oksenberg JR, Hauser SL. Molecular and genetic aspects of multiple sclerosis. In: Rosenberg RN, Prusiner SB, DiMauro S, Barchi RL, eds. The molecular and genetic basis of neurological disease. 2nd ed Boston: Butterworth–Heinemann, 1997:631-60.
116. Genain CP, Cannella B, Hauser SL, Raine CS. Identification of autoantibodies associated with myelin damage in multiple sclerosis. Nat Med 1999;5:170-5.
117. Lucchinetti C, Brück W, Parisi J, Scheithauer B, Rodriguez M, Lassmann H. Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. Ann Neurol 2000;47:707-17.
118. Benson MD. Amyloidosis. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. The metabolic and molecular bases of inherited disease. 7th ed. Vol. 3. New York: McGraw-Hill, 1995:4159-91.
119. United Nations. World population prospects: 1998 revision. Vol. 2. The sex and age distribution of the world population. New York: United Nations Department of Economic and Social Affairs Population Division, 1999:1-833.